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ACOUSTIC IDENTIFICATION OF TWELVE SPECIES OF ECHOLOCATING BAT BY DISCRIMINANT FUNCTION ANALYSIS AND ARTIFICIAL NEURAL NETWORKS

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Summary

We recorded echolocation calls from 14 sympatric species of bat in Britain. Once digitised, one temporal and four spectral features were measured from each call. The frequency–time course of each call was approximated by fitting eight mathematical functions, and the goodness of fit, represented by the mean-squared error, was calculated. Measurements were taken using an automated process that extracted a single call from background noise and measured all variables without intervention. Two species of *Rhinolophus* were easily identified from call duration and spectral measurements. For the remaining 12 species, discriminant function analysis and multilayer back-propagation perceptrons were used to classify calls to species level. Analyses were carried out with and without the inclusion of curve-fitting data to evaluate its usefulness in distinguishing among species. Discriminant function analysis achieved an overall correct classification rate of 79 % with curve-fitting data included, while an artificial neural network achieved 87 %. The removal of curve-fitting data improved the performance of the discriminant function analysis by 2 %, while the performance of a perceptron decreased by 2 %. However, an increase in correct identification rates when curve-fitting information was included was not found for all species. The use of a hierarchical classification system, whereby calls were first classified to genus level and then to species level, had little effect on correct classification rates by discriminant function analysis but did improve rates achieved by perceptrons. This is the first published study to use artificial neural networks to classify the echolocation calls of bats to species level. Our findings are discussed in terms of recent advances in recording and analysis technologies, and are related to factors causing convergence and divergence of echolocation call design in bats.

Key words: echolocation, species identification, bat, discriminant function analysis, artificial neural network, call shape.

Introduction

Effective monitoring of echolocation calls is vital in many studies of the ecology and conservation of bats (Fenton, 1997). The identification of individual bats, or members of the same or different species, from their echolocation calls has proved difficult because of technological and analytical limitations, the ability to obtain truly representative calls under controlled conditions and the extreme flexibility in call design exhibited by many species. The echolocation calls of different species are also not equally conspicuous; several produce low-amplitude calls that are not easily detected (e.g. Fenton and Bell, 1981). Echolocation calls also vary both inter- and intraspecifically because of the influence of acoustic clutter, morphology, age and foraging strategy (e.g. Bogdanowicz et al., 1999; Griffin et al., 1960; Jensen and Miller, 1999; Jones, 1999; Jones et al., 1992; Jones and Kokurewicz, 1994; Kalko and Schnitzler, 1989, 1993; Masters et al., 1995; Obrist, 1995; Rydell, 1990). All these factors influence the structure of calls and thus the ability of researchers to distinguish between them. Other factors influencing successful identification include recording quality and methodology (Lawrence and Simmons, 1982; Pye, 1992, 1993; Parsons, 1996, 1998; Surlykke et al., 1993) and the analysis method and variables used to characterise calls (Parsons and Obrist, 2000).

Practically all published quantitative acoustic studies investigating individual and species identification of bats have used multivariate statistics, especially discriminant function analysis (DFA; e.g. Krusic and Neefus, 1996; Lance et al., 1996; Murray et al., 1999; Neefus and Krusic, 1995; Obrist, 1995; Parsons, 1997; Vaughan et al., 1997; Zingg, 1990). Recently, two new techniques have been applied to classify the emitter of an echolocation call. Obrist et al. (2000) developed a system that digitises bat calls in real time and uses synergetic pattern recognition algorithms to perform species identification. The system compares incoming digitised calls with a stored reference library of prototyped calls from known species. Real-time recording systems are ideal because all the information is retained in the recorded signal. Burnett and Masters (1999) used a self-organising map, a type of artificial neural network (ANN), to estimate the number of bats that produced a number of echolocation calls on the basis of

temporal and spectral measurements. Self-organising maps can be used to identify intrinsic features contained in an input data set and use these to estimate natural groupings within data sets. Neural networks have been successfully applied in other areas of bioacoustics, such as target classification from echoes by cetaceans (Au, 1994; Au et al., 1995) and bats (Altes, 1995; Dror et al., 1995; Wotton and Jenison, 1997), and for classifying the vocalisations of marine mammals (Deecke et al., 1999; Murray et al., 1998). In general, the ability of neural networks to solve very complex acoustic problems has been promising. However, they have never been used to identify the echolocation calls of bats to species level.

Multilayer perceptrons, a popular class of ANN, can be 'taught' to recognise patterns so that, when presented with previously unseen data, they can classify them correctly. Learning is achieved by modifying synaptic weights between units of the network, termed neurones. Back-propagation networks, a form of multilayer perceptron, use a gradient-descent algorithm to minimise the error caused by misclassifications during training (Carling, 1992; Haykin, 1999; Rumelhart et al., 1986). In this way, they are fundamentally different from DFA, which does not utilise any error-minimisation algorithm. In general, back-propagation networks give reasonable results when presented with inputs they have never seen before. The ability of back-propagation networks to generalise makes it possible to train a network on a representative set of input/target pairs and obtain reliable classifications without training the network on all possible pairs.

The measurements traditionally used to describe the echolocation calls of bats, such as duration, start frequency, end frequency and the frequency with most energy, have also been used in species identification studies. These measurements are static in that they do not describe how a call changes over time. Several studies have used mathematical functions to approximate the frequency–time course of echolocation calls (Masters et al., 1991; Masters and Raver, 2000; Parsons et al., 1997). The purpose of these studies was to study the signal-processing capabilities of bats. However, these functions can also be used as templates to describe the 'shape' of calls in terms of their deviation from each of the functions.

In this paper, we present the results of a study in which the echolocation calls of 14 species of bat were recorded in the field using either time-expansion or high-speed sampling direct to computer. Calls were then analysed digitally, and the measurements were used to classify calls to species level. We measured temporal and spectral features of calls and used eight mathematical functions to give an estimate of the frequency–time course, or 'shape', of calls from 12 species (calls produced by the two *Rhinolophus* species are easily identified using only temporal and spectral measurements). Calls were described in terms of their approximation to the functions. Automated analysis methods were developed to minimise input from the investigators, making this study more objective than those requiring measurements by hand. Discriminant function analysis and back-propagation neural networks were used to classify calls, and the results were compared. Both analyses were carried out with and without the inclusion of shape information to judge the usefulness of shape in separating the calls of different species. This study is the first to include a measure of spectral shape and ANNs in an acoustic system for identifying species of echolocating bat. We follow Jones and Barratt (1999) in our naming of the two cryptic species of pipistrelle as *Pi. pipistrellus* (45 kHz phonic type of *Pi. pipistrellus*) and *Pi. pygmaeus* (55 kHz phonic type of *Pi. pipistrellus*) (Jones and van Parijs, 1993).

Materials and methods

Recording methods and call analysis

We recorded search-phase echolocation calls (as defined by Griffin, 1958) outside known roosts (all species), on release from the hand after capture by harp-trap or mist-net (*Myotis bechsteinii*, *M. brandtii*, *M. daubentonii*, *M. mystacinus*, *M. nattererii* and *Plecotus auritus*) or at foraging sites where species and individual bats were identified unambiguously (*Nyctalus noctula*; Table 1). All recordings were made in 1998 and 1999 between May and October. To avoid recording the same bats over several nights, only calls made on one night were analysed. We recorded calls as far away from the roost entrance as possible while still being able to ensure that the bats recorded had emerged from that roost. Recordings were usually made on-axis as the bat flew towards the microphone, which was housed on a tripod approximately 1.2 m above the ground. Rarely was the bat at the same height above the ground as the microphone. Calls made as bats were released from the hand were recorded as far from the release point as possible, although usually less than 2 m from the microphone. With the exception of *Barbastella barbastellus* and *Nyctalus leisleri*, we recorded each species at several distinct geographic locations. We recorded a total of 698 calls. Of the bats known to breed in Britain, only *Pipistrellus nathusii* and *Plecotus austriacus* were not recorded.

We used two different recording methods. Initially, we used an UltraSound Advice (USA; UltraSound Advice, London, UK) S-25 bat detector (frequency response of microphone 20–120 kHz ±3 dB) linked to a USA portable ultrasonic processor (PUSP). The PUSP, when triggered by the user as a bat flew past the microphone, digitised a 2 s sequence of sound. Sequences were sampled at 448 kHz with eight-bit precision and time-expanded by ten times before being recorded to a Sony WD6 Professional Walkman. Calls were digitised to computer using the BatSound software (Pettersson Elektronik AB, Uppsala, Sweden) at a sampling rate of 44.1 kHz (effective rate 441 kHz) using the standard sound card contained in a Toshiba Satellite Pro laptop computer (model 4080XCDT, Toshiba of Europe, London, UK). From early 1999, we digitised echolocation calls directly to the laptop computer (i.e. not time-expanded). Using the S-25 bat detector, coupled to a National Instruments PCMCIA format analogue-to-digital (A/D) conversion board (model AI-16E-4; National Instruments, Austin, TX, USA), 5 s sequences of sound were

Table 1. *Species recorded in this study including the number of distinct geographic locations where recordings were made, the number of calls recorded per species and the situation in which the bats were recorded*

	Common name	Location	Number of calls	Situation
Barbastella barbastellus (Schreber, 1774)	Barbastelle	1	33	r
Eptesicus serotinus (Schreber, 1774)	Serotine bat	5	56	r
Myotis bechsteinii (Kuhl, 1818)	Bechstein's bat	5	25	r, h
M. brandtii (Eversmann, 1845)	Brandt's bat	2	50	r, h
M. daubentonii (Kuhl, 1819)	Daubenton's bat	3	24	r, h
M. mystacinus (Kuhl, 1819)	Whiskered bat	4	37	r, h
M. nattereri (Kuhl, 1818)	Natterer's bat	4	82	r, h
Nyctalus leisleri (Kuhl, 1818)	Leisler's bat	1	80	r
N. noctula (Schreber, 1774)	Noctule bat	3	90	r, f
Pipistrellus pipistrellus (Schreber, 1774)	Common (45 kHz) pipistrelle	8	36	r
P. pygmaeus (Leach, 1825)	Soprano (55 kHz) pipistrelle	3	96	r
Plecotus auritus (Linnaeus, 1758)	Brown long-eared bat	4	32	r, h
Rhinolophus ferrumequinum (Schreber, 1774)	Greater horseshoe bat	2	24	r
R. hipposideros (Bechstein, 1800)	Lesser horseshoe bat	3	33	r
Total			698	

r, flying away from a roost; h, on release from the hand; f, foraging sites.

acquired. Calls were digitised, when the system was triggered by the user, using BatSound, at a sampling rate of 500 kHz, with 12-bit precision.

Using BatSound, we chose for further analysis a single call that had the highest signal-to-noise ratio without being overloaded from each sequence. Calls were transferred to MatLab v5.3 (Mathworks, Natick, MA, USA) for further analysis. A mid-pass (10–150 kHz) tenth-order Butterworth filter was applied to the signals and the envelope of each created using a Hilbert transform of the waveform. Envelopes were subsequently scaled between 0 and 1. Using the point where the envelope rose above and then subsequently dropped below an arbitrary threshold value, the call was removed from the rest of the signal. All calls were extracted from the background noise using the same arbitrary threshold value (0.003). Call duration (Durtn) was defined as the duration of the extracted waveform. The frequency with most energy (F-maxE) was measured from a power spectrum. As the duration of calls varied both inter- and intraspecifically, the number of points used for the Fast Fourier Transformation (FFT) was set to be the closest power of two larger than the total number of points in the call. A Hamming window was applied to the call before the FFT was calculated. The resulting power spectrum was smoothed to 1024 points, allowing the frequency with most energy from calls of different durations to be compared, giving a frequency resolution of 430 Hz and 488 Hz for signals digitised at 441 kHz and 500 kHz respectively. No call was shorter than 1024 points. The frequency–time course of the harmonic with most energy was recreated by dividing the call into a series of 56-point segments. We calculated power spectra for each segment (after the application of a Hamming window) using a zero-padded 1024-point FFT, and the frequency with most energy was calculated within each power spectrum. This gave a frequency *versus* time reconstruction of the call at 127 µs and 112 µs intervals when digitised at 441 kHz or 500 kHz respectively (Fig. 1). Each reconstruction was checked by eye to ensure that there were no obvious errors. The start and end frequencies (F-start and F-end respectively) of the call were taken to be the first and last values from the reconstructed call respectively. The frequency at half the duration (F-centre) was also taken from the reconstruction.

With the exception of the calls of *Pl. auritus*, the harmonic with most energy within each call was always measured. At the beginning of a *Pl. auritus* call, most energy is usually in the fundamental, after which it can switch to the second harmonic. For the sake of consistency in our measurements, we chose to measure all variables, except the frequency with

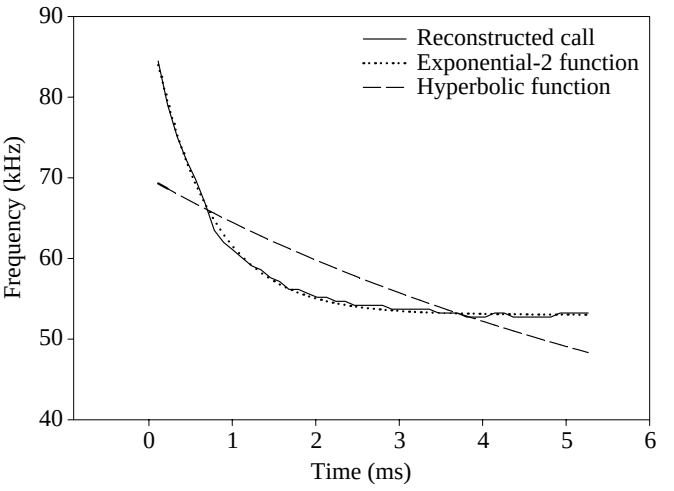

Fig. 1. Reconstruction of the frequency–time course of a call recorded from *Pipistrellus pygmaeus*. Also shown are the approximations to the true frequency–time course by exponential-2 and hyperbolic functions

most energy, from the fundamental of *Pl. auritus* calls. The frequency with most energy was measured across all harmonics for all species.

Modelling of frequency sweeps

We fitted eight mathematical curve functions to the reconstructed frequency–time course of calls using a non-linear regression procedure in MatLab's statistics toolbox (version 2.2; Fig. 1). Three of these curves (hyperbolic, logarithmic, exponential-1) are the same as those used by Masters et al. (1991) and Parsons et al. (1997). For all equations $f(t)$ is the frequency of the fundamental at time t after the start of the call, f_0 is the starting frequency at t=0, and f_1 is the asymptotic frequency approached as t becomes large. Where a or c is present, they represent decay constants. The linear sweep function is given by:

$$f(t) = f_0 - (f_0 - f_1)t, \quad (1)$$

the exponential-1 decay is given by:

$$f(t) = f_0 \left(\frac{f_1}{f_0} \right)^t , \quad (2)$$

the hyperbolic sweep function is given by:

$$f(t) = \frac{f_0 f_1}{f_1 + (f_0 - f_1)t^2} \quad (3)$$

and the power-1 sweep function is given by:

$$f(t) = f_0 - (f_0 - f_1)t^2 . \quad (4)$$

For all four of the above functions, when $f_0 > f_1$, the frequency sweep of the call is downwards. The exponential-2 decay is given by:

$$f(t) = \frac{f_0}{f_0 - af_1} \left[(f_0 - f_1) \left(\frac{af_1}{f_0} \right)^t + (1 - a)f_1 \right] . \quad (5)$$

As a approaches f_0/f_1, the call approaches a linear frequency sweep; for $a > (f_0/f_1)$, the sweep is convex, and for a=0, the curvature is infinite and describes a call with a vertical frequency-modulated component followed by a constant-frequency component. The exponential-2 function is the only one used in this study capable of describing a constant-frequency component in a call. The logarithmic-sweep function is given by:

$$f(t) = f_0 - (f_0 - f_1) \frac{\log_e(1 + at)}{\log_e(1 + a)} . \quad (6)$$

The parabolic sweep function is given by:

$$f(t) = f_0 - (1 + a)(f_0 - f_1)t + a(f_0 - f_1)t^2 . \quad (7)$$

When a=0, the call sweep is linear, and when a<0, the call sweep is convex. The power-3 sweep function is given by:

$$f(t) = f_0 - (f_0 - f_1) \frac{(1 + at)^c - 1}{(1 + a)^c - 1} . \quad (8)$$

The power-3 function is the most flexible used in this study and can approximate linear, power-1, logarithmic and parabolic functions.

Design and training of the neural network

To test the ability of ANNs to classify echolocation calls to species level, we trained multilayer perceptrons using a back-propagation algorithm with momentum (e.g. Haykin, 1999; Rumelhart et al., 1986), epoch training and adaptive learning (Vogl et al., 1988) using the neural network toolbox (toolbox version 3.01) of Matlab version 5.3.

We trained a number of different networks depending on the classification task required (e.g. to genus level, *Myotis* only, all species). In all cases, the inputs to the networks were the five temporal and spectral call variables and eight mean-squared errors (MSEs) from the curve-fitting analysis. The network outputs were the species emitting the calls to be classified. Either one or two hidden layers were used, and the number of neurones in each was varied between five and 20 in steps of five. The momentum constant was varied between 0.1 and 0.9 in steps of 0.1. The most suitable architecture was defined as that giving the highest correct identification rate. Networks were trained using 50 % of the input data set. Prior to being split, data were randomised within each species to remove any effect of recording equipment, year of recording, recording site or geographic variation in call structure. Each variable in the training data set was transformed by dividing through by the largest value, making each vary between 0 and 1. The largest value for each variable was also used to standardise its corresponding variable in the test data set. The performance of networks during training was represented by the root-mean-squared (RMS) error of observed *versus* expected outputs. To achieve reasonable performance, the training algorithm was often repeated for many thousands of epochs until this RMS error was reduced to some arbitrarily selected level (0.05 was found to give reasonable results). After training, the remaining 50 % of the dataset was used to test the networks independently. The network architecture producing the highest overall correct identification rate was then rerun 30 times, each time using different initial random weights and biases for each neurone to ensure that the highest classification rate had been achieved. The best-performing of these networks was used to discriminate between calls.

Results

Description of echolocation calls and curve fits

Both *B. barbastellus* and *N. noctula* produce two distinct types of call (Fig. 2) based on systematic differences in call durations and spectral variables (Table 2). The two call types were not specified prior to the discriminant function and neural network analyses being carried out, as this would have required subjective classification. However, they have been separated here to facilitate a more accurate description of each species' vocal repertoire. The echolocation calls recorded showed a great deal of flexibility both within and among species

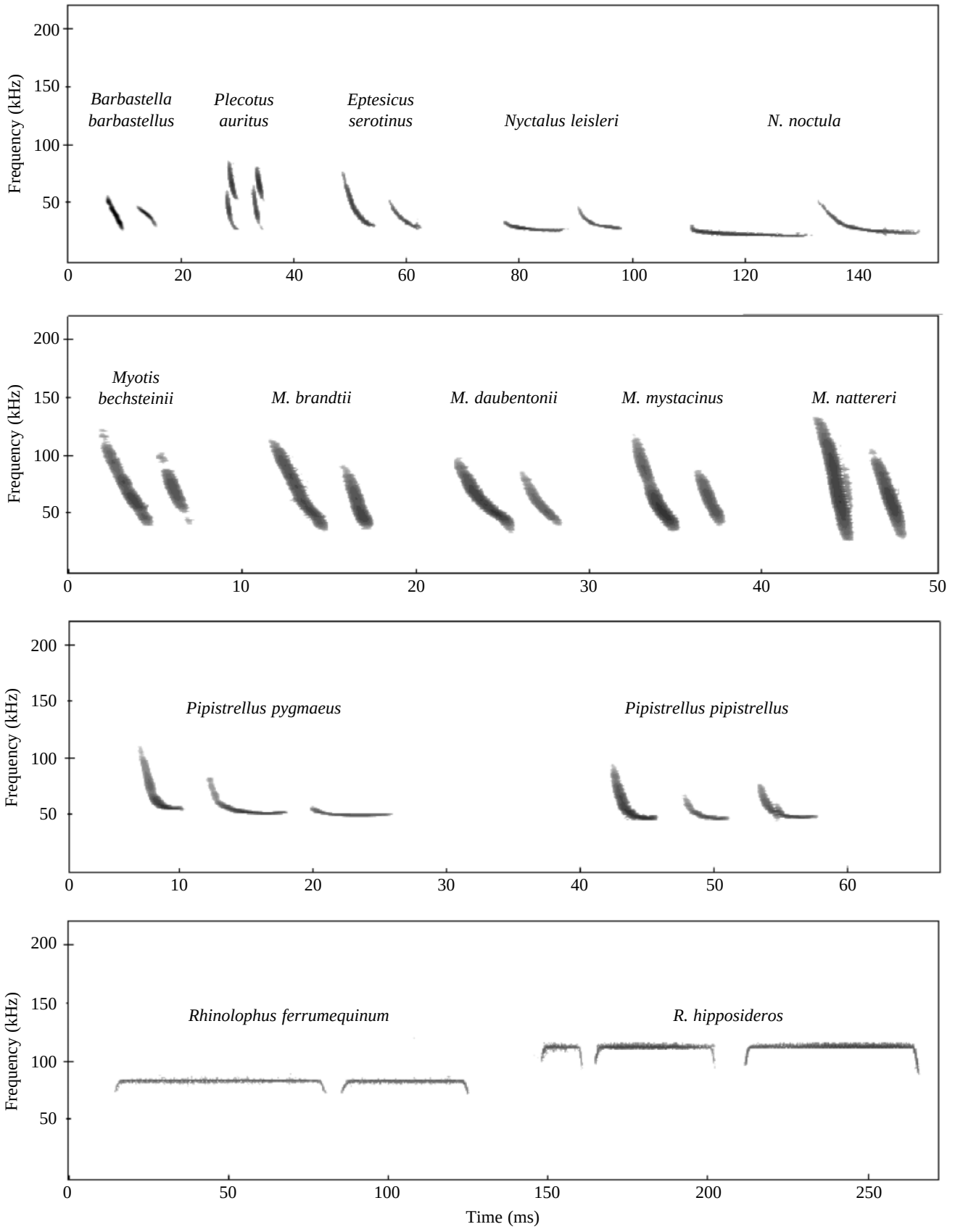

Fig. 2. Spectrograms of 2–3 echolocation calls recorded from the 14 species of bat to indicate variability. Type-1 and type-2 calls for *Barbastella barbastellus* and *Nyctalus noctula* are shown. Spectrograms were created using a 512-point FFT with Hamming windowing. Gaps between successive pulses do not indicate true interpulse intervals.

Table 2. *Summary statistics for echolocation calls recorded from free-flying bats*

	N	Locations	Duration (ms)	F-start (kHz)	F-end (kHz)	F-maxE (kHz)	F-centre (kHz)
Barbastella barbastellus 1	21	1	2.50±0.13 (24.61)	37.98±1.03 (12.46)	29.72±0.51 (7.87)	33.18±0.72 (9.92)	33.65±0.77 (10.50)
B. barbastellus 2	12	1	3.76±0.22 (20.51)	45.54±0.75 (5.68)	30.51±1.21 (13.76)	39.09±1.04 (9.20)	39.80±0.64 (5.60)
Eptesicus serotinus	56	5	6.71±0.29 (31.89)	61.54±0.95 (11.58)	28.26±0.29 (7.74)	33.67±0.54 (11.95)	35.00±0.55 (11.70)
Myotis bechsteinii	25	5	2.16±0.08 (19.05)	111.96±3.04 (13.60)	42.24±0.81 (9.56)	73.04±1.76 (12.05)	77.21±1.27 (8.20)
M. brandtii	50	2	3.84±0.11 (20.07)	101.05±1.21 (8.46)	35.82±0.43 (8.48)	55.21±0.87 (11.13)	62.38±0.98 (11.07)
M. daubentonii	24	3	2.79±0.17 (29.38)	88.20±1.05 (5.85)	36.46±1.08 (14.56)	54.89±0.77 (6.84)	59.50±1.07 (8.82)
M. mystacinus	37	4	2.36±0.09 (21.96)	102.68±1.95 (11.58)	39.93±0.84 (12.82)	57.58±1.26 (13.35)	68.80±1.65 (14.59)
M. nattereri	82	4	3.35±0.19 (50.48)	121.44±1.37 (10.20)	30.80±0.61 (17.95)	64.64±1.67 (23.45)	79.33±1.86 (21.18)
Nyctalus leisleri	80	1	7.60±0.27 (31.98)	58.63±1.52 (23.25)	28.38±0.34 (10.64)	31.00±0.43 (12.47)	32.05±0.50 (14.08)
N. noctula 1	43	3	11.53±0.43 (24.31)	47.19±1.29 (17.95)	25.50±0.34 (8.86)	26.92±0.38 (9.36)	27.51±0.42 (10.00)
N. noctula 2	47	3	13.09±0.38 (20.02)	31.25±0.79 (17.30)	21.58±0.28 (8.88)	21.98±3.53 (6.79)	22.01±0.23 (7.23)
Pipistrellus pipistrellus	36	8	4.78±0.19 (24.41)	77.03±2.02 (15.75)	46.29±0.34 (4.37)	46.74±0.29 (3.76)	47.52±0.32 (4.06)
P. pygmaeus	96	3	5.53±0.09 (15.80)	86.69±1.33 (15.05)	52.71±0.17 (3.11)	52.86±0.18 (3.35)	53.51±0.21 (3.93)
Plecotus auritus	32	4	2.12±0.09 (25.18)	55.14±1.19 (12.16)	29.91±1.04 (19.63)	51.96±2.29 (24.92)	40.65±1.61 (22.34)
Rhinolophus ferrumequinum	24	2	51.50±2.56 (24.34)	69.48±0.42 (2.96)	67.94±0.80 (5.80)	82.08±0.11 (0.66)	82.22±0.12 (0.71)
R. hipposideros	33	3	41.70±1.48 (20.35)	98.19±0.87 (5.12)	96.33±1.37 (8.15)	110.98±0.24 (1.23)	110.95±0.25 (1.28)

Values are presented as means ± S.E.M.

Values in parentheses are coefficients of variation.

N, number of calls per individual; F-start, start frequency; F-end, end frequency; F-maxE, frequency with the most energy; F-centre, frequency with the most energy at half the duration of the call.

For *B. barbastellus* and *N. noctula*, values are shown for the two distinct call types recorded for these species.

(Table 2). In general, call duration was the most variable parameter measured, with most species having coefficients of variation greater than 20 %. The remainder of the parameters were equally variable, with the exception of those measured from the *Rhinolophus* spp. whose specialised method of echolocation makes their calls highly conserved. Among species, mean call durations varied from 2.12 ms for *Pl. auritus* to 51.5 ms for *R. ferrumequinum*. The calls of *M. nattereri* had the highest start frequency (121.4 kHz). Of the five *Myotis* spp. recorded, only those of *M. daubentonii* started on average below 100 kHz. The type-2 calls of *N. noctula* had the lowest start frequency (31.3 kHz). *R. hipposideros* produced calls whose frequency with maximum energy was the highest (111.0 kHz), while the type-2 calls of *N. noctula* was the lowest, a result mirrored by the frequency at half the duration of the call (111.0 kHz and 22.0 kHz for *R. hipposideros* and *N. noctula* type-2 respectively).

On average, the frequency–time course of calls produced by individual *Eptesicus serotinus*, *M. brandtii*, *M. nattereri*, *N. leisleri* and *N. noctula* (type-1 calls) and the calls of the two pipistrelle species were best described by the exponential-2 sweep function (*M. nattereri* equal with log; Table 3). The calls of *B. barbastellus* (type-1 and type-2 calls) were best described by the logarithmic function. The parabolic function described the calls of *Pl. auritus* best, while the power-3 sweep function described the calls of *M. bechsteinii*, *M. daubentonii*, *M. mystacinus* and *N. noctula* (type-2 calls) best. The exponential-1, hyperbolic, linear and power-1 sweep functions

Table 3. *Average mean-squared errors for the different functions fitted to the frequency–time courses of echolocation calls*

	Exp-1	Exp-2	Hyper	Lin	Log	Para	Pow-1	Pow-3
Barbastella barbastellus 1	0.37±0.07 (82.82)	0.23±0.04 (82.61)	0.41±0.09 (96.07)	0.37±0.06 (78.79)	0.22±0.04 (84.73)	0.23±0.04 (81.67)	0.31±0.07 (101.72)	0.39±0.18 (208.33)
B. barbastellus 2	1.09±0.41 (131.10)	0.37±0.10 (97.80)	1.46±0.48 (113.50)	0.79±0.35 (150.87)	0.36±0.12 (113.24)	0.38±0.10 (90.30)	1.45±1.13 (270.45)	5.69±2.82 (171.53)
Eptesicus serotinus	5.83±0.40 (51.43)	0.39±0.06 (122.74)	2.23±0.22 (75.04)	11.51±0.70 (45.58)	1.02±0.10 (71.90)	1.12±0.14 (93.94)	1.53±0.13 (65.60)	1.21±0.46 (278.61)
Myotis bechsteinii	4.30±0.75 (87.49)	0.94±0.17 (90.42)	18.31±2.27 (61.87)	3.77±0.81 (107.89)	0.91±0.15 (84.46)	0.99±0.19 (95.07)	0.94±0.15 (78.83)	0.81±0.16 (94.70)
M. brandtii	3.40±1.30 (270.86)	1.00±0.14 (97.15)	8.60±1.94 (159.77)	10.31±0.99 (67.94)	1.20±0.24 (139.93)	1.13±0.10 (60.86)	1.58±0.16 (69.23)	1.61±0.32 (135.39)
M. daubentonii	2.45±0.27 (53.49)	1.62±0.21 (64.12)	4.34±0.74 (83.28)	5.58±0.54 (47.63)	1.46±0.21 (70.54)	1.93±0.23 (58.18)	1.53±0.21 (67.13)	1.41±0.21 (72.46)
M. mystacinus	3.18±0.71 (135.20)	1.36±0.19 (83.43)	10.04±1.82 (110.46)	6.09±0.75 (75.14)	1.37±0.19 (85.62)	1.45±0.20 (82.75)	1.52±0.23 (91.80)	1.05±0.11 (58.11)
M. nattereri	27.61±3.02 (99.17)	1.90±0.15 (73.55)	68.38±5.90 (78.10)	10.50±0.92 (79.73)	1.90±0.15 (74.02)	2.15±0.19 (80.67)	1.95±0.15 (70.95)	2.03±0.32 (140.85)
Nyctalus leisleri	9.89±0.66 (59.57)	0.38±0.03 (62.11)	5.83±0.41 (63.23)	14.46±0.97 (59.86)	1.36±0.10 (65.39)	2.78±0.22 (70.99)	1.49±0.11 (63.95)	4.16±2.10 (446.05)
N. noctula 1	5.48±0.54 (64.23)	0.38±0.04 (63.99)	3.69±0.33 (59.29)	7.31±0.76 (67.87)	0.67±0.07 (72.92)	1.68±0.17 (68.19)	0.72±0.07 (66.02)	2.21±0.92 (273.21)
N. noctula 2	1.36±0.21 (104.63)	0.37±0.03 (57.94)	1.23±0.18 (97.50)	1.48±0.24 (110.26)	0.39±0.04 (68.29)	0.66±0.07 (72.53)	0.39±0.04 (68.79)	0.27±0.03 (70.02)
Pipistrellus pipistrellus	21.83±2.51 (68.95)	0.31±0.04 (86.25)	17.27±1.77 (61.55)	25.89±3.16 (73.30)	4.87±0.65 (79.72)	6.03±0.77 (76.45)	4.93±0.65 (79.67)	16.23±6.54 (241.94)
P. pygmaeus	27.87±1.69 (59.58)	0.42±0.07 (172.48)	22.86±1.28 (55.04)	32.50±2.16 (65.06)	6.93±0.48 (67.63)	7.75±0.50 (62.59)	7.01±0.49 (67.95)	20.86±4.81 (222.13)
Plecotus auritus	5.15±1.81 (199.24)	2.69±1.83 (385.59)	5.26±2.85 (305.96)	8.25±2.86 (196.31)	3.14±2.03 (366.05)	2.53±1.53 (342.58)	3.34±2.09 (353.82)	3.79±2.30 (338.68)

Values are presented as means ± S.E.M.

Values in parentheses are coefficients of variation.

Exp-1, exponential-1; Exp-2, exponential-2; Hyper, hyperbolic; Lin, linear; Log, logarithmic; Para, parabolic; Pow-1, power-1; Pow-3, power-3.

For *B. barbastellus* and *N. noctula*, values are shown for the two distinct call types recorded for these species.

on average did not provide the best description of the frequency–time course of any species' calls.

Large intraspecific variation in MSEs was apparent, with coefficients of variation for some species being nearly 400 % (Table 3). This high degree of variability was often caused by poor fits of one or more functions to only one or two calls from each species. Expressing the function best describing the calls of a species based on average MSEs may thus be misleading. Therefore, we also calculated the number of calls best described by each function with a species (Table 4). The majority of calls produced by *M. brandtii*, *M. nattereri* and *N. leisleri* and the type-1 calls produced by *B. barbastellus* are now best described by the power-3 function, and the calls of *M. bechsteinii* are almost equally well described by the parabolic and power-1 sweep functions.

Statistical and discriminant function analysis – with shape information

None of the 13 variables measured from echolocation calls conformed to the multivariate normal distribution (Box's *M*-test, F=17.065, P<0.0001). However, DFA is relatively robust to deviations from normality, which are likely to reduce performance slightly (Dillon and Goldstein, 1984). Examination of the covariance matrices showed that they were heterogeneous and that transformation of the data did not reduce heterogeneity, nor did it reduce deviation of the data from normality. Therefore, quadratic discriminant functions were calculated in all analyses (Dillon and Goldstein, 1984) using untransformed data. Cross-validation was used in all DFAs.

Discriminant function analysis of the 13 variables measured from 641 calls from 12 species gave an overall correct classification rate of 79 % (Fig. 3A). Random classification of the data would produce a correct classification rate of 8 %. Multivariate analysis of variance (MANOVA) showed that discrimination of the data was significantly different from random (Wilk's λ=0.00029, F=59.105, P<0.001) and that 64 % of the variation was explained by the first discriminant

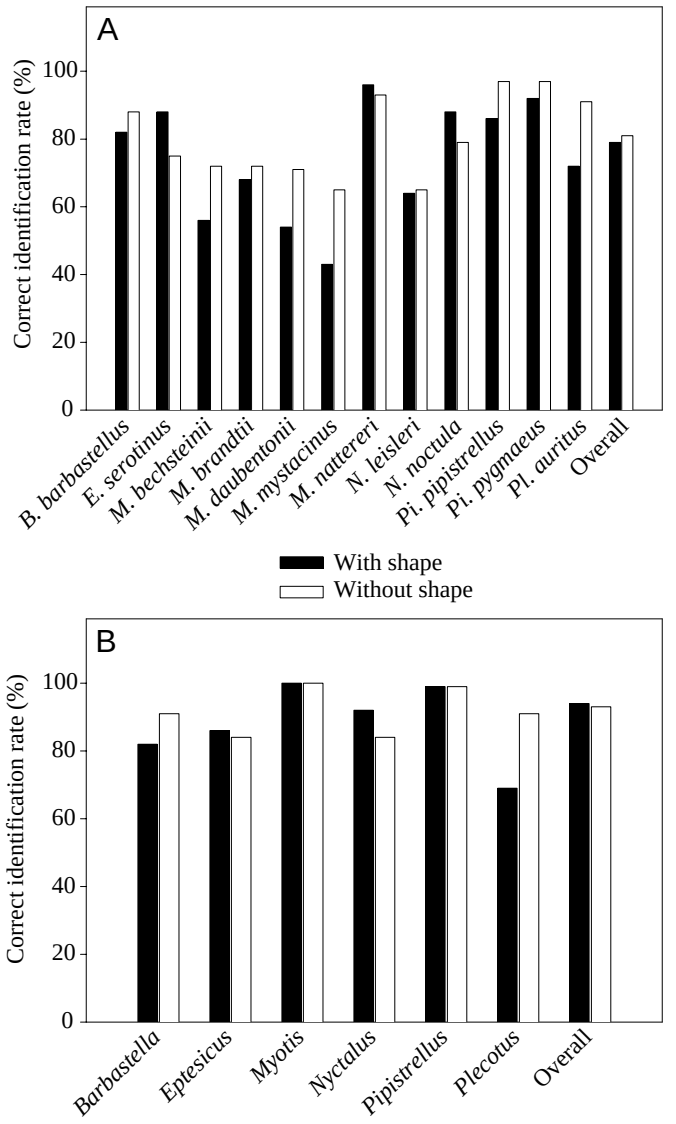

Fig. 3. Correct identification rates from discriminant function analysis (DFA) with shape data included or not included in the data sets. Analyses were carried out at (A) species and (B) genus level.

function. The first three discriminant functions accounted for 99 % of the variation in the data. The highest correct discrimination rate was achieved for *M. nattereri*, with 96 % of recorded calls correctly identified. Identification rates of *Pi. pygmaeus* calls were also high, with 92 % correctly identified. Four species (*E. serotinus*, *N. noctula*, *B. barbastellus* and *Pi. pygmaeus*) had over 80 % of their recorded calls identified correctly. With the exception of *Pl. auritus* (correct identification rate 72 %), all the remaining species, four of which belong to the genus *Myotis*, had less than 70 % of their calls identified correctly. The calls of *M. mystacinus* were the most difficult to classify, with only 43 % correctly identified.

Wilks' λ statistics were used to determine the contribution each variable made to the ability of DFA to classify calls. Of the 13 variables measured, the five most important were temporal and spectral characteristics, with F-end being the most important (Table 5). The MSE for the eight curve functions fitted to the calls were the least important for discriminating among species.

DFA was also used to classify calls to genus level (Fig. 3B). The overall correct classification rate of calls, which was significantly different from random (MANOVA, Wilks' λ=0.0030, F=109.316, P<0.001), was 94 % with the calls recorded from *Myotis* spp. unambiguously identified. The first discriminant function accounted for 62 % of the variation in the data, while the first five functions accounted for 100 % of the variation. The calls from *Pipistrellus* spp. and *Nyctalus* spp. had correct classification rates of over 90 %. Of the three monospecific genera in this study, only the calls of *Plecotus* sp. were correctly classified at a rate of less than 80 %. Correct identification rates for the monospecific genera were very similar to those achieved by the species-level discriminant function analysis. Only one call recorded from *Pipistrellus* spp. was incorrectly identified, being classified as belonging to *Nyctalus* spp. Similarly, two calls emitted from *Nyctalus* spp. were incorrectly identified as belonging to *Pipistrellus* spp. In both these cases, misidentification appeared to be due to similarities in MSE values rather than temporal and spectral features. Temporal and spectral features were the most important for discriminating among genera (Table 5). The relative importance of the temporal and spectral measurements was the same as for the all-species DFA with the exception of F-centre, which was the second most important in the genus-level DFA (compared with the third most important in the all-species DFA).

DFA was used to classify calls within each of the multispecific genera. Overall, correct classification rates of 72 %, 82 % and 91 % were achieved for *Myotis* spp., *Nyctalus* spp. and *Pipistrellus* spp. respectively (Table 6), all of which were significantly different from random (*Myotis* spp., Wilks' λ=0.08250, F=12.634, P<0.001; *Nyctalus* spp., Wilks' λ=0.61789, F=4.329, P<0.001; *Pipistrellus* spp., Wilks' λ=0.21194, F=32.893, P<0.001). For the *Myotis* spp., 100 % of the variation in the data was explained by the first four discriminant functions, while all the variation in both the *Nyctalus* spp. and *Pipistrellus* spp. data was explained by their respective first discriminant functions. Correct identification rates for individual *Myotis* spp. were identical to those achieved by the all-species DFA. This result is not surprising because calls produced by *Myotis* spp. were only ever confused with the calls of other *Myotis* spp. by the all-species DFA. However, the correct classification rates achieved by the within-genera DFA for calls emitted by *N. leisleri*, *N. noctula* (Table 6), *Pi. pipistrellus* and *Pi. pygmaeus* (Table 6) were equal to or higher than those achieved by the all-species DFA. MSE values were more important in discriminating between the calls of *Myotis* spp. than those of *Nyctalus* spp. and *Pipistrellus* spp., with hyperbolic and exponential-1 MSEs being the third and fourth most important respectively (Table 5). The five temporal and spectral features were the most important for distinguishing between calls recorded from *Nyctalus* spp. and *Pipistrellus* spp. F-start, F-maxE and F-end

Table 4. *Number of echolocation calls from each species categorised by best function*

	Exp-1	Exp-2	Hyper	Lin	Log	Para	Pow-1	Pow-3
Barbastella barbastellus 1	0 (0)	1 (5)	0 (0)	0 (0)	3 (14)	2 (10)	7 (33)	8 (38)
B. barbastellus 2	0 (0)	0 (0)	0 (0)	0 (0)	8 (67)	1 (8)	2 (17)	1 (8)
Eptesicus serotinus	0 (0)	30 (54)	0 (0)	0 (0)	1 (2)	7 (13)	0 (0)	18 (32)
Myotis bechsteinii	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (40)	9 (36)	6 (24)
M. brandtii	0 (0)	7 (14)	0 (0)	0 (0)	0 (0)	20 (40)	2 (4)	21 (42)
M. daubentonii	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	5 (21)	5 (21)	13 (54)
M. mystacinus	0 (0)	3 (8)	0 (0)	0 (0)	0 (0)	11 (30)	9 (24)	14 (38)
M. nattereri	0 (0)	4 (5)	0 (0)	0 (0)	10 (12)	11 (13)	22 (27)	35 (43)
Nyctalus leisleri	0 (0)	28 (35)	0 (0)	0 (0)	0 (0)	5 (6)	1 (1)	46 (58)
N. noctula 1	0 (0)	19 (44)	0 (0)	0 (0)	4 (9)	0 (0)	1 (2)	19 (44)
N. noctula 2	0 (0)	4 (9)	0 (0)	0 (0)	0 (0)	0 (0)	12 (26)	31 (66)
Pipistrellus pipistrellus	0 (0)	21 (58)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	15 (42)
P. pygmaeus	0 (0)	75 (78)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	20 (21)
Plecotus auritus	0 (0)	8 (25)	0 (0)	0 (0)	2 (6)	13 (41)	5 (16)	4 (13)
Total	0 (0)	201 (31)	0 (0)	0 (0)	28 (4)	86 (13)	75 (12)	251 (39)

Best function is defined as the curve function whose mean-squared error is the lowest for each call.

Values in parentheses are the numbers of best function calls expressed as a percentage of the total number of calls for that species.

Exp-1, exponential-1; Exp-2, exponential-2; Hyper, hyperbolic; Lin, linear; Log, logarithmic; Para, parabolic; Pow-1, power-1; Pow-3, power-3.

For *B. barbastellus* and *N. noctula*, values are shown for the two distinct call types recorded for these species.

were the most important variables for distinguishing between the calls of *Myotis* spp., *Nyctalus* spp. and *Pipistrellus* spp. respectively.

Statistical and discriminant function analysis – without shape information

To test the contribution made by the inclusion of signal shape to the ability of recorded calls to be classified, DFAs were carried out using only temporal and spectral characteristics measured from individual calls. The overall correct classification rate for all species was higher than when shape information was included (81 %; Fig. 3). Multivariate analysis of variance (MANOVA) showed that discrimination of the data was significantly different from random (Wilks' λ=0.0021, F=146.031, P<0.001). Correct identification rates for individual species were higher when shape information was removed for all species except *E. serotinus*, *M. nattereri* and *N. noctula*. At the genus level, the overall correct classification rate was reduced (93 %) when shape information was removed despite all individual genera except *Eptesicus* and *Nyctalus* having higher correct identification rates. MANOVA showed that discrimination was significantly different from random (Wilks' λ=0.01402, F=202.124, P<0.001). The first three discriminant functions from the all-species and genus-level analyses described 98 % of the variation in the data.

Classification of calls by artificial neural networks – with shape information

Back-propagation networks were trained using all 13 variables measured from 12 species. The best architecture consisted of a network with two hidden layers, each containing 20 neurones. Using this architecture, an overall correct classification rate of 87 % was achieved (Fig. 4A). Random classification would give an identification rate of 8.3 %. Perfect classification was achieved for *B. barbastellus* and both species of pipistrelle. Correct identification rates for calls produced by

Table 5. *Relative importance of each call parameter in discriminating between the echolocation calls of different species by discriminant function analysis (DFA)*

	Wilks' λ				
Variable	All species	Genus	*Myotis* spp.	*Nyctalus* spp.	*Pipistrellus* spp.
F-end	0.126	0.213	0.507	0.585	0.270
F-start	0.139	0.221	0.495	0.599	0.897
F-centre	0.147	0.214	0.689	0.539	0.370
F-maxE	0.200	0.262	0.763	0.527	0.293
Duration	0.221	0.345	0.791	0.550	0.890
Hyperbolic MSEs	0.494	0.812	0.584	0.748	0.959
Linear MSEs	0.571	0.620	0.865	0.641	0.980
Exponential-1 MSEs	0.596	0.779	0.686	0.672	0.973
Parabolic MSEs	0.624	0.649	0.885	0.778	0.975
Logarithmic MSEs	0.702	0.718	0.936	0.723	0.959
Power-1 MSEs	0.722	0.738	0.938	0.701	0.96
Power-3 MSEs	0.907	0.912	0.989	0.986	0.996
Exponential-2 MSEs	0.922	0.931	0.896	1.000	0.994

The lower the value for Wilks' λ, the more important the variable.

F-end, end frequency; F-start, start frequency; F-centre, frequency with the most energy at half the duration of the cell; F-maxE, frequency with most energy; MSE, mean-squared error.

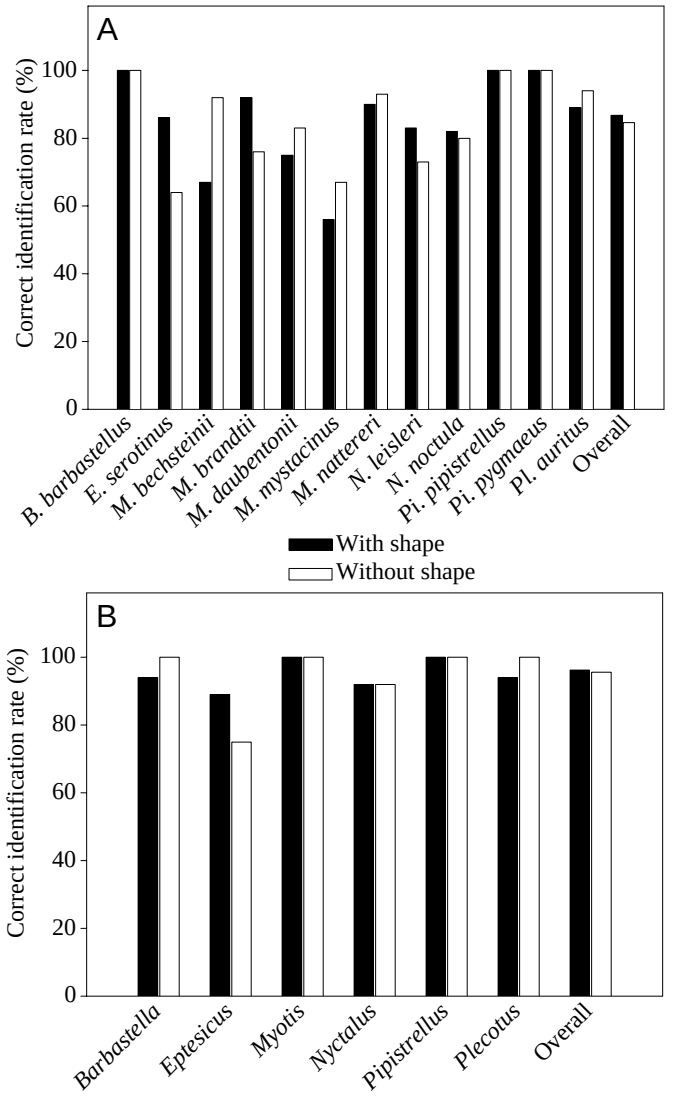

Fig. 4. Correct identification rates for artificial neural networks (ANNs) trained to identify calls with shape data included or not included in the training and testing data sets. Networks were trained to identify calls to (A) species and (B) genus level.

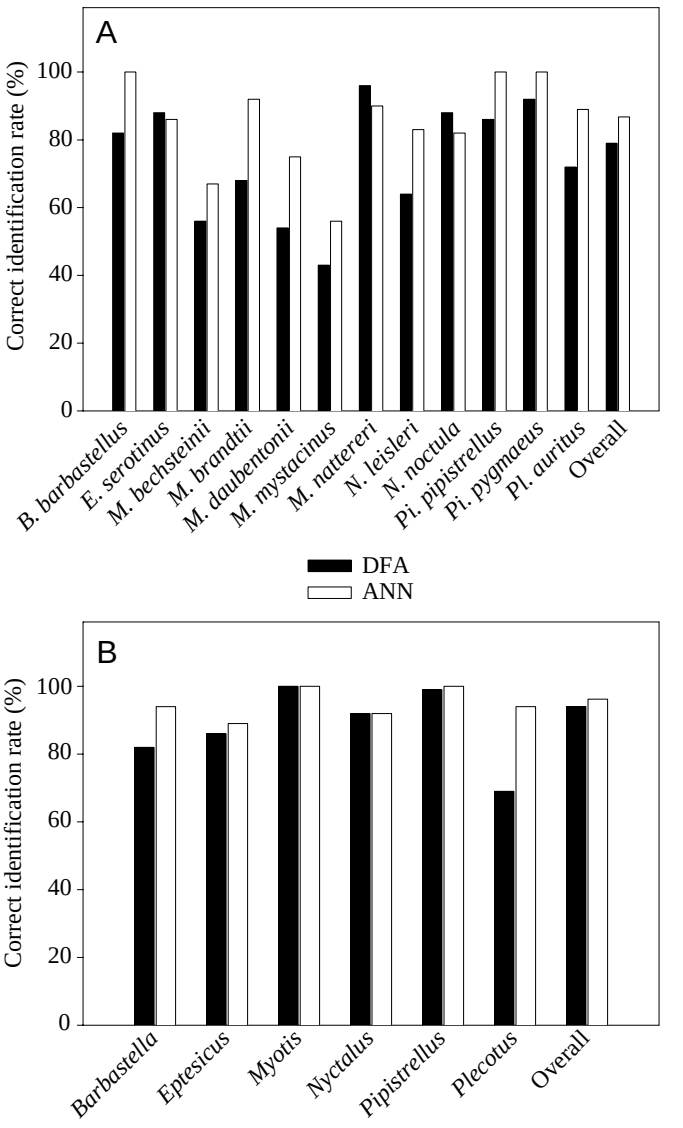

Fig. 5. A comparison of correct identification rates achieved by artificial neural networks (ANNs) and discriminant function analyses (DFAs) with shape information. Comparisons are made at (A) species and (B) genus level.

M. brandtii and *M. nattereri* were equal to or greater than 90 %, while correct identification rates for *E. serotinus*, *N. leisleri*, *N. noctula* and *Pl. auritus* were greater than 80 %. Correct identification rates for calls produced by *M. daubentonii*, *M. bechsteinii* and *M. mystacinus* were 75 %, 67 % and 56 % respectively. For the majority of species, correct identification of recorded calls was equal to or higher than that using the equivalent DFA (Fig. 5A). The only exceptions were *E. serotinus*, *M. nattereri* and *N. noctula*.

A network whose architecture consisted of one hidden layer containing 20 neurones was trained to classify calls to genus level (Fig. 4B). The overall correct identification rate for this network was 96 %. Calls from species within the genera *Myotis* and *Pipistrellus* were identified without error. Only calls from *Eptesicus* sp. had a correct identification rate of less than 90 %. However, the identification rate was 3 % higher than for calls classified to the species level by the previous network. The identification rates for the other two genera represented by only one species increased by 5 % for *Pl. auritus*, but decreased by 6 % for *B. barbastellus*, compared with results from the network trained to discriminate between all species. For all genera, correct identification of recorded calls was equal to or higher than that achieved by the equivalent DFA (Fig. 5B).

Three genus-specific networks were trained to classify calls from bats within each of the genera containing multiple species. The first network, trained to classify calls produced only by *Myotis* spp., consisted of two hidden layers, each containing 20 neurones. The overall successful classification rate achieved by this network was 82 % (Table 7). The calls of *M. nattereri* were classified with a success rate of 90 %. Calls were confused with all those of all other *Myotis* spp. except *M.*

Table 6. *Results from three separate discriminant analyses used to classify the calls of bats belonging to the genera* Myotis, Nyctalus *or* Pipistrellus

	True group				
Classified as	*M. bechsteinii*	*M. brandtii*	*M. daubentonii*	*M. mystacinus*	*M. nattereri*
Genus *Myotis*					
M. bechsteinii	14	2	1	8	0
M. brandtii	1	34	3	3	1
M. daubentonii	0	3	13	7	0
M. mystacinus	4	6	7	16	2
M. nattereri	6	5	0	3	79
Total *N*	25	50	24	37	82
N correct	14	34	13	16	79
% Correct	56	68	54	43	96

	True group	
	N. leisleri	*N. noctula*
Genus *Nyctalus*		
N. leisleri	60	10
N. noctula	20	80
Total *N*	80	90
N correct	60	80
% Correct	75	89

	True group	
	P. pipistrellus	*P. pygmaeus*
Genus *Pipistrellus*		
P. pipistrellus	31	7
P. pygmaeus	5	89
Total *N*	36	96
N correct	31	89
% Correct	86	93

The overall correct classification rates were 72 %, 82 % and 91 % respectively.

Table 7. *Results from three neural networks trained to categorise the calls of bats belonging to the genera* Myotis, Nyctalus *or* Pipistrellus

	True group				
Classified as	*M. bechsteinii*	*M. brandtii*	*M. daubentonii*	*M. mystacinus*	*M. nattereri*
Genus *Myotis*					
M. bechsteinii	9	2	0	1	2
M. brandtii	0	21	0	1	1
M. daubentonii	1	1	9	1	0
M. mystacinus	2	1	3	14	1
M. nattereri	0	0	0	1	37
Total *N*	12	25	12	18	41
N correct	9	21	9	14	41
% Correct	75	84	75	78	90

	True group	
	N. leisleri	*N. noctula*
Genus *Nyctalus*		
N. leisleri	39	1
N. noctula	1	43
Total *N*	40	44
N correct	39	43
% Correct	98	98

	True group	
	P. pipistrellus	*P. pygmaeus*
Genus *Pipistrellus*		
P. pipistrellus	18	0
P. pygmaeus	0	48
Total *N*	18	48
N correct	18	48
% Correct	100	100

daubentonii. Of calls recorded from *M. brandtii*, 84 % were correctly classified. The calls of *M. brandtii* were confused with those of all the other *Myotis* spp. except *M. nattereri*. The correct identification rate of calls recorded from *M. mystacinus* was 78 %, while 75 % of calls recorded from *M. bechsteinii* and *M. daubentonii* were correctly identified. With the exception of calls recorded from *M. brandtii*, the correct identification rate of calls recorded from *Myotis* spp. using this network was equal to or higher than the correct identification rate achieved by the all-species network.

The second genus-specific network was trained to identify calls recorded from *N. leisleri* and *N. noctula* (Table 7). The

best network architecture consisted of two hidden layers, each containing 10 neurones each. Only one call from each species was misidentified, giving correct identification rates for each, and an overall correct identification rate, of 98 %. A third network, trained to identify the calls from *Pi. pipistrellus* and *Pi. pygmaeus*, consisted of one hidden layer containing five neurones. This network correctly identified all the calls recorded from both species (Table 7). The correct identification rate of calls recorded from *Nyctalus* spp. and *Pipistrellus* spp. was equal to or higher than that achieved by the all-species network.

Classification of calls by artificial neural network – no shape information

A neural network was trained to identify calls recorded from 12 species of bat using only the five temporal and spectral features, i.e. without the MSE data. The best network architecture, which consisted of one hidden layer containing 10 neurones, achieved an overall correct identification rate of 85 % (Fig. 4A). Identification rates were lower compared with the network trained using shape information for *E. serotinus*, *M. brandtii*, *N. leisleri* and *N. noctula*. However, the removal of shape information increased the identification rates for *M. bechsteinii*, *M. daubentonii*, *M. mystacinus*, *M. nattereri* and *Pl. auritus*. For the majority of species, correct identification of recorded calls was equal to or higher than with the equivalent DFA. The only exception was *E. serotinus*. A second network, trained to classify calls to genus level only, had a best network architecture consisting of two hidden layers each containing 20 neurones. This network was equal to or better at discriminating calls from individual genera than the equivalent network trained and tested using shape information (Fig. 4B), with four of the six genera having correct identification rates of 100 %. Because of the low correct identification rate achieved for calls from *Eptesicus* sp., the overall correct identification rate was lower than when shape information was included. For all but one of the genera (*Eptesicus*), correct identification of recorded calls was equal to or higher than the equivalent DFA.

Discussion

Plasticity in echolocation call design

The demands of certain acoustic tasks have caused convergence in signal design within and among species of bat. As acoustic clutter increases, calls become shorter and more broadband, and pulse repetition rate increases (e.g. Kalko and Schnitzler, 1993; Rydell, 1990). Bats using echolocation calls with a low duty cycle must make these changes to avoid overlap between outgoing pulses and echoes from objects in close proximity and to resolve the exact position of the clutter-producing background (Jensen and Miller, 1999; Kalko and Schnitzler, 1989, 1993; Simmons and Stein, 1980). Call parameters vary similarly as a bat approaches a target, except that, after an initial increase in signal bandwidth, calls become progressively less broadband with increasing proximity to the object (Kalko, 1995; Masters et al., 1991; Simmons et al., 1979). Morphology also influences call design and can cause convergence in call design between morphologically similar species. Call frequency correlates negatively with forearm length and body mass, while call duration scales positively with body mass in several genera (Bogdanowicz et al., 1999; Jones, 1996, 1999). Age has also been shown to have an effect on the echolocation calls of bats independent of morphology (Jones et al., 1992; Jones and Kokurewicz, 1994; Masters et al., 1995). The constraints on signal design we have mentioned do not mean, however, that all bats must produce the same calls. For example, both *M. bechsteinii* and *Pl. auritus* prefer wooded areas for foraging, glean prey from surfaces and include a significant proportion of tympanate Lepidoptera in their diet (Entwistle et al., 1996; Vaughan, 1997). However, *Pl. auritus* produces calls with a different design from those of *M. bechsteinii*. At the same time, the present study and many others have shown that some closely related species use very similar call designs, perhaps because of phylogenetic constraints. The most obvious example of this occurs in the calls from members the genus *Myotis* found in Britain.

The majority of temporal and spectral measurements taken from calls in the present study agree well with those reported previously in the literature (Ahlén, 1981; Britton and Jones, 1999; Jensen and Miller, 1999; Jones and Rayner, 1989; Jones, 1995; Kalko and Schnitzler, 1989; Konstantinov and Makarov, 1981; Rydell et al., 1999; Vaughan et al., 1997; Waters et al., 1995; Zingg, 1988, 1990). Where differences do occur, they are usually small and can be easily explained by plasticity in call design. Average values for measurements taken from *Myotis* spp. match those from the literature less well and are difficult to explain in terms of acoustic clutter, foraging ecology or morphological scaling. In particular, frequency measurements (but not F-start and bandwidth) appear to be higher than those previously reported. The differences in mean values are due to the influence of measurements taken from calls recorded after bats had been released from the hand. Although call durations are not excessively short, changes in the structure of calls recorded in this situation resemble those seen in other species, including *Myotis* spp., when flying in enclosed rooms or on release from the hand (Britton and Jones, 1999; Parsons, 1998; Waters and Jones, 1995). However, both the DFAs and ANNs were exposed to calls from *Myotis* spp. recorded in free flight and on release from the hand, and calls from both situations were misclassified.

Previous studies have reported the use of alternating call types by both *N. noctula* and *B. barbastellus*. When high above the ground, *N. noctula* alternates between long, almost constant-frequency calls and shorter more frequency-modulated calls (Ahlén, 1981; Zbinden, 1989). The use of a long narrowband call when flying in very open environments would allow bats to detect objects such as large insects at greater distances and may also allow them to maintain acoustic contact with the ground, thus providing an important reference

point. The more frequency-modulated call would provide detailed information on the bats' surroundings and allow the detection of prey items such as small dipterans that have been shown to be an important dietary component (Jones, 1995). The long narrowband call may not be used when bats fly close to the ground as this situation represents a high-clutter environment (Jensen and Miller, 1999).

The function of the two alternating call types in *B. barbastellus* is less clear. Ahlén (1981) analysed echolocation calls recorded indoors and outside and noted two pulse types, emitted alternately, the descriptions of which match our type-1 and type-2 calls. Konstantinov and Makarov (1981) described a slightly convex frequency-modulated sweep similar in structure to our type-2 calls, but sweeping down to a lower frequency, with a greater bandwidth. A neuronal audiogram showed that hearing was most sensitive in the frequency range between 20 and 30 kHz (Konstantinov and Makarov, 1981). Although the calls they describe fall within this range, neither the calls we recorded nor those of Ahlén (1981) do. Our recordings show that type-2 calls are of much lower amplitude than type-1 calls, a phenomenon also noted by Ahlén (1981), who also recorded sequences in which bats emitted only low-amplitude calls. In all our sequences, bats produced only type-1 calls or alternated between type-1 and type-2 calls. However, given the recording situation (near a tree roost), it is unlikely that bats were actively foraging, and the function of alternating call types remains unclear.

Shape of echolocation calls

Masters et al. (1991) and Parsons et al. (1997) both used iterative curve-fitting to describe the frequency–time course of bat echolocation calls relative to predefined curve functions. However, the present study is the first to include this information for species identification. It is clear from temporal and spectral measurements and from the results of the curve-fitting analysis that the structure of calls is highly variable. In general, the more flexible the function, in terms of its ability to change its rate of frequency modulation, the better it was at describing calls. The inclusion of shape information degraded the ability of DFA to classify calls correctly. Examination of λ statistics consistently showed that the MSE values from the curve functions were the least suitable for discriminating among groups. Their inclusion in the DFA may, therefore, have made the task of constructing effective discriminant functions more difficult by acting as noise, thus obscuring the true signal in the data. The inclusion of shape information had a mixed effect on the classification of calls, at the genus and species level, by the ANNs. The size of the networks required to achieve the best results was larger when shape information was included, indicating that the task of classifying the data was more complicated. Unfortunately, it is much more difficult to examine ANNs because of their distributed form of non-linearity and high connectivity. The use of hidden layers also makes it difficult to visualise the learning process. ANNs essentially represent a 'black box', and this is one of their major deficiencies.

Discriminant analysis versus *neural networks for species identification*

DFA has been widely used to classify the species or individual bat producing an echolocation call. In most studies, both temporal and spectral features have been measured from either time-expanded or frequency-divided signals. Obrist (1995) and Vaughan et al. (1997) analysed time-expanded calls and obtained correct identification rates varying from 12 to 97 %. Zingg (1990), analyzing frequency-divided signals, achieved correct classification rates between 72 % and 99 %. Lance et al. (1996) also analysed frequency-divided calls from individuals on release from the hand, not during free flight. The results of their study showed that only two species, *Pipistrellus subflavus* and *Lasiurus seminolus*, could be correctly identified with a confidence greater than 90 %. For the other five species, correct identification rates were all below 50 %. Krusic and Neefus (1996) noted particular problems in identifying the frequency-divided calls of *Myotis* spp. Although they could identify all non-*Myotis* species with an accuracy of 100 %, identification rates for *Myotis* spp. varied between 42 and 87 %. However, at the genus level, identification of *Myotis* spp. was possible with an accuracy of 97 %.

Neural networks have been used in a variety of classification tasks in biological sonar research (e.g. Au, 1994; Au et al., 1995; Deecke et al., 1999; Dror et al., 1995; Murray et al., 1998; Wotton and Jenison, 1997). Burnett and Masters (1999) used ANNs to identify bats from their echolocation calls. They used a back-propagation network and self-organising map to classify the echolocation calls of individual *E. fuscus*. The network was able to identify 50 % of individuals correctly, the same success rate as that achieved by a DFA. The results obtained from the self-organising map increased as more bats were included in the analysis. Parsons (2000) also used a back-propagation network to classify the calls of two species of bat in New Zealand. Time-expanded calls were easily identified but heterodyned calls were not. The classification task required by our networks is far more complex.

To classify calls to species level using a single analysis, given the number of species in the study and the number of variables measured per call, is not a trivial task, especially given the highly variable nature of the data. By using a hierarchical classification system, in which calls were classified to genus and species level by separate functions or networks, it was thought that the ability of both DFAs and ANNs to classify species correctly would improve. In the case of the DFA, the improvement was only small, with classification rates of *N. leisleri* increasing by the greatest amount. The classification rates of the other species from multispecific genera either stayed the same or increased slightly. Identification rates for *Myotis* spp. did not improve under the hierarchical system because calls were only ever misclassified as belonging to other *Myotis* spp. Unexpectedly, the correct classification rates for *B. barbastellus*, *E. serotinus* and *Pl. auritus* decreased when classifications were made to genus level rather than species level. It is clear that the success

of a hierarchical classification system of DFAs is dependent on the species to be analysed and the nature of any misclassifications. The use of hierarchical ANNs was much more effective. Identification rates increased or stayed the same for all species except two. This highlights the power of ANNs because the pattern of misclassifications from the all-species analysis was very similar to that of the all-species DFA.

At every systematic level, the ANNs outperformed their equivalent DFA. DFA uses series of functions that best separate the groups and then classifies each data point in turn. However, the neural network we employed in this study used an error back-propagation algorithm (Rumelhart et al., 1986) based on the error-correction learning rule. Error back-propagation learning consists of two passes through the different layers of the network: a forward pass and a backward pass. In the forward pass, inputs are presented to the network and a signal passes through the various layers, resulting in a set of actual responses from the network. During the forward pass, the synaptic weights of the neurones in the network are fixed. During the backward pass, these synaptic weights are adjusted to make the actual response of the network match the desired response. The use of a network with hidden neurones, i.e. neurones that are not part of the input or output layers, means that the network can learn complex tasks by extracting progressively more meaningful features from the input data. Therefore, given the complexity of species identification, it is not surprising that the ANNs outperformed the DFAs.

We chose not to use interpulse interval as a variable despite the fact that studies have shown it to be important for separating species acoustically (e.g. Vaughan et al., 1997). This was done because the use of interpulse interval requires the identity of two successive calls to be known *a priori*. We also analysed calls from some species in situations with, or simulating (as in the case of hand-released animals), large amounts of acoustic clutter where most species tend to converge on a similar call design, a short frequency-modulated sweep. Therefore, the success rates we achieved are even more remarkable.

Concluding remarks

Although the results of this study are extremely promising and demonstrate that ANNs may prove to be a useful technique for acoustic identification of bats, they should not be generalised. The correct identification rates we achieved are particular to the species studied, the methods with which their calls were recorded and analysed, the variables we chose to measure and the situations (particularly the amount of acoustic clutter present) in which we recorded the bats. However, the results of this study show that ANNs can be used to identify the echolocation calls of bats to species level and that their performance is consistently better than that of the equivalent DFA. Although most bat species emit very different echolocation calls, the need for flexibility means that species will inevitably produce calls that are very similar to those of another species. This means that not every call produced by a bat will be easily discriminated from those of other species. However, providing that an adequate acoustic inventory is constructed for a species, that the call variables that best separate species are measured and that the technique best able to deal with the variability inherent to the data is used, many of the calls can be identified unambiguously.

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